

Influence of Elevated Carbon Dioxide on Water Relations of Soybeans¹

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ABSTRACT

Soybean (*Glycine max* L. Merrill cv 'Bragg') plants were grown in pots at six elevated atmospheric CO₂ concentrations and two watering regimes in open top field chambers to characterize leaf xylem potential, stomatal resistance and conductance, transpiration, and carbohydrate contents of the leaves in response to CO₂ enrichment and water stress conditions. Groups of plants at each CO₂ concentration were subjected to water stress by withholding irrigation for 4 days during the pod-filling stage.

Under well watered conditions, the stomatal conductance of the plants decreased with increasing CO₂ concentration. Therefore, although leaf area per plant was greater in the high CO₂ treatments, the rate of water loss per plant decreased with CO₂ enrichment. After 4 days without irrigation, plants in lower CO₂ treatments showed greater leaf tissue damage, lower leaf water potential, and higher stomatal resistance than high CO₂ plants. Stomatal closure occurred at lower leaf water potentials for the low CO₂ grown plants than the high CO₂ grown plants. Significantly greater starch concentrations were found in leaves of high CO₂ plants, and the reductions in leaf starch and increases in soluble sugars due to water stress were greater for low CO₂ plants. The results showed that even though greater growth was observed at high atmospheric CO₂ concentrations, lower rates of water use delayed and, thereby, prevented the onset of severe water stress under conditions of low moisture availability.

The net transfer of carbon to the atmosphere through deforestation and the burning of fossil fuels in recent decades (2, 20, 23) has created a need to understand possible effects of our current energy and land use policies on future agricultural productivity. Dry matter production by crop plants depends directly on CO₂ concentration not only as substrate for photosynthesis but also as regulator of stomatal aperture and, therefore, of transpiration rate (8). Since moisture availability restricts crop growth more than all other environmental factors combined (4, 10), increased economy of water use should have a widespread positive impact on yield potentials.

Attempts to study the effects of elevated CO₂ on crop plants

in the field using open air fumigation techniques have largely failed to overcome the problems of CO₂ dispersal by turbulent air (1). However, in the open top field chamber (16), plants may be exposed continuously to elevated CO₂ under conditions close to field conditions. In the present experiment, soybean plants were grown in pots in open top chambers at various CO₂ concentrations and subjected to withholding of irrigation during the critical pod-filling period. Our purpose was to document the influence of CO₂ concentration on water use and on the response of soybeans to water stress characterized in terms of leaf water potential, stomatal resistance and conductance, transpiration, and carbohydrate accumulation.

MATERIALS AND METHODS

Soybean plants (*Glycine max* L. Merrill cv 'Bragg') were grown from seed in open field chambers (16) at different atmospheric CO₂ concentrations. Each chamber consisted of a cylindrical aluminum frame (3 m in diameter × 2.4 m in height) covered with PVC film Roll-A-Glass² with a 45° frustum attached at the top. Air with the desired CO₂ concentration was supplied day and night at 1.06 m³ s⁻¹ through perforations in the inner wall of the lower half of the chamber.

There were two open (no chamber) plots and two replicates of each of six CO₂ treatments within chambers with mean seasonal daytime CO₂ concentrations (±1 SD) of 332 ± 11, 428 ± 30, 534 ± 37, 623 ± 46, 772 ± 52, and 910 ± 65 μl l⁻¹. A LSI-11 microcomputer was used to automatically control CO₂ sampling from each plot.

Each plot contained 20 plants grown singly in 16.5-L pots in 2:1:1 mixture by volume of sandy clay loam soil:sand:Metro Mix 220 (W. R. Grace and Co). Except during the drying cycle imposed during early pod-filling, plants were irrigated when tensiometers reached 25 to 35 centibars. A nitrogen-free nutrient solution (7) was applied to each pot weekly throughout the growing period. During early pod-filling, half the plants in each plot were subjected to a drying cycle by withholding water, while the other half (controls) were watered daily. Conditions were warm and sunny throughout the drying and recovery cycle. During the stress experiment, stomatal resistance (conductance) and transpiration rates were measured daily between 1300 and 1530 h EST with Li-Cor steady state porometers. Readings were taken on the abaxial surface of the middle leaflet of the third trifoliate leaf from the top of the main stem of all 20 plants

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during the stress period and after rewatering. Resistance data for well watered plants (Fig. 1b) were collected in a similar fashion but on an overcast afternoon in early reproductive phase when fluctuations in light and temperature were relatively low. For estimates of water use, 10 control plants per CO₂ treatment were weighed in their pots morning and evening for 3 d. Values, therefore, represent average change in weight per pot per min from about 0600 to 1500 h EST. Mid-afternoon xylem potential measurements were made with a pressure bomb on four plants per stress treatment per CO₂ treatment using the third through sixth leaves from the top of the plant, in balanced rotation among treatments and days. Carbohydrate content was determined on six control and six stressed plants per CO₂ treatment. Leaf discs were collected from 1300 to 1500 h EST on the 4th d after watering from the third, fourth, and fifth trifoliates from the top of the main stem, combined, quick frozen on dry ice, lyophilized, and ground in 80% ethanol. Total ethanol-soluble sugars were acid hydrolyzed and determined according to Nelson (11) and Somogyi (19) against sucrose standards. Starch concentrations were determined on the residue from the ethanol extraction after amyloglucosidase incubation using the Nelson (11) and Somogyi (19) assay and glucose standards.

RESULTS

Repeated measurements of stomatal conductance of well watered soybean plants made with a steady state porometer have

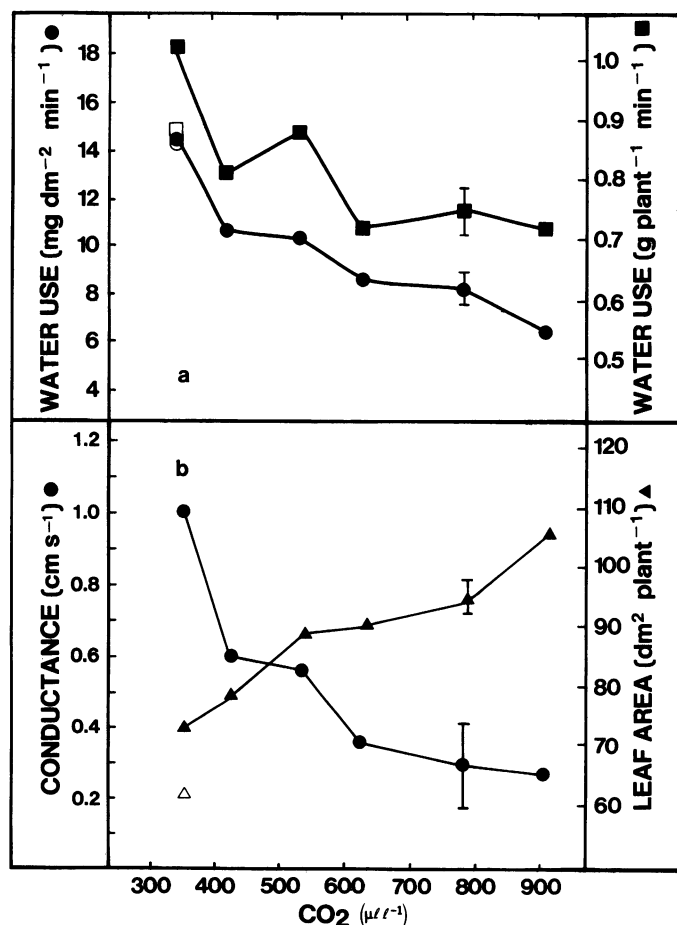


FIG. 1. Water use per plant and per unit leaf area (a) and leaf area per plant and stomatal conductance of the third trifoliolate leaf (b) in an ambient plot (open symbols) and six CO₂ treatments in field chambers (solid symbols). Conductance for ambient plot = 22. Bars, SE from ANOVA; $n = 20$ for stomatal conductance; $n = 10$ for other variables.

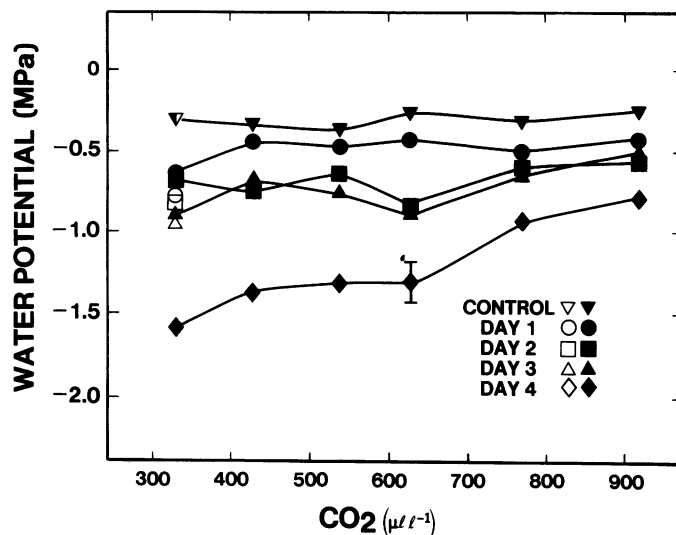


FIG. 2. Mean afternoon leaf water potentials for 4 d after withholding water from soybean plants at early pod fill. Open symbols = ambient plots (no chambers). Bar at day 4, SE from ANOVA; $n = 4$ for stressed treatments; Control points, mean values for well watered plants for days 0 through 4 ($n = 20$).

shown significant decreases at high CO₂ concentrations (Fig. 1b). In order to know to what extent increased leaf area under these conditions would counteract low conductance per unit leaf area in canopy transpiration, water use per plant was determined gravimetrically for several days. Transpiration per plant was found to decrease with increasing CO₂ concentration (Fig. 1a) in spite of increased leaf area per plant (Fig. 1b). Expressed on a unit leaf area basis, a similar decrease in water use was observed, with less variability (Fig. 1a).

The effects of CO₂ enrichment on leaf stomatal aperture were advantageous under conditions of water stress imposed during early pod filling. Figure 2 shows the gradual decrease in water potential during the 4 d after watering ceased across the range of CO₂ treatments. The leaf water potential of plants grown in ambient CO₂ outside the chambers declined at the greatest rate and reached -1.65 MPa, the lowest value in any of the CO₂ treatments (open symbols). These and plants with small increments of added CO₂ showed severe wilting and injury of the lower leaves, which did not recover after rewatering, although the upper leaves regained the water potential value of the control plants within 24 h after rewatering (data not shown). Water potentials of plants grown in high CO₂ atmospheres decreased at lower rates and remained higher at the end of the stress treatment than plants in low CO₂ treatments, and damage to lower leaves was not as extensive. For well watered plants, there were no significant differences in water potential due to CO₂.

Stomatal resistance measurements (Fig. 3) and transpiration measurements (Fig. 4) also reflected the advantage of high CO₂-grown plants in a situation of limited moisture availability. Early in the week, leaves of the 910 μl l⁻¹ treatment clearly had higher resistance (Fig. 3), with transpiration being correspondingly low (Fig. 4). On day 1, transpiration was twice as high at the low as at the intermediate CO₂ levels, with a corresponding difference in resistance. On the afternoon of day 4 of the stress, leaves of plants at 332 μl l⁻¹ gave the highest resistance readings, the 910 μl l⁻¹ treatment the lowest, and the 623 μl l⁻¹ treatment an intermediate response (Fig. 3), and all the plants transpired at nearly the same very low rate (Fig. 4). Resistance did not return to the control range until the 2nd d after rewatering, and transpiration also remained low for 2 d (Figs. 3 and 4) in spite of the more rapid recovery of water potential. After recovery, leaves of stressed plants in all three treatments showed transpiration rates

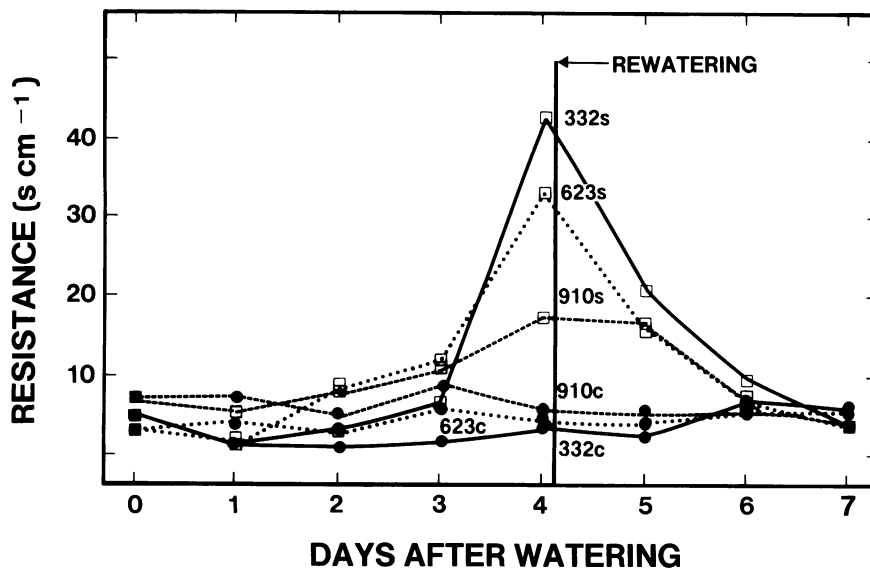


FIG. 3. Mean stomatal resistance on d 4 of the stress treatment measured on the third trifoliolate leaf of soybeans grown at low ($332 \mu\text{l l}^{-1}$), intermediate ($623 \mu\text{l l}^{-1}$), and high ($910 \mu\text{l l}^{-1}$) CO₂ concentrations in field chambers. s, stressed; c, controls; $n = 10$.

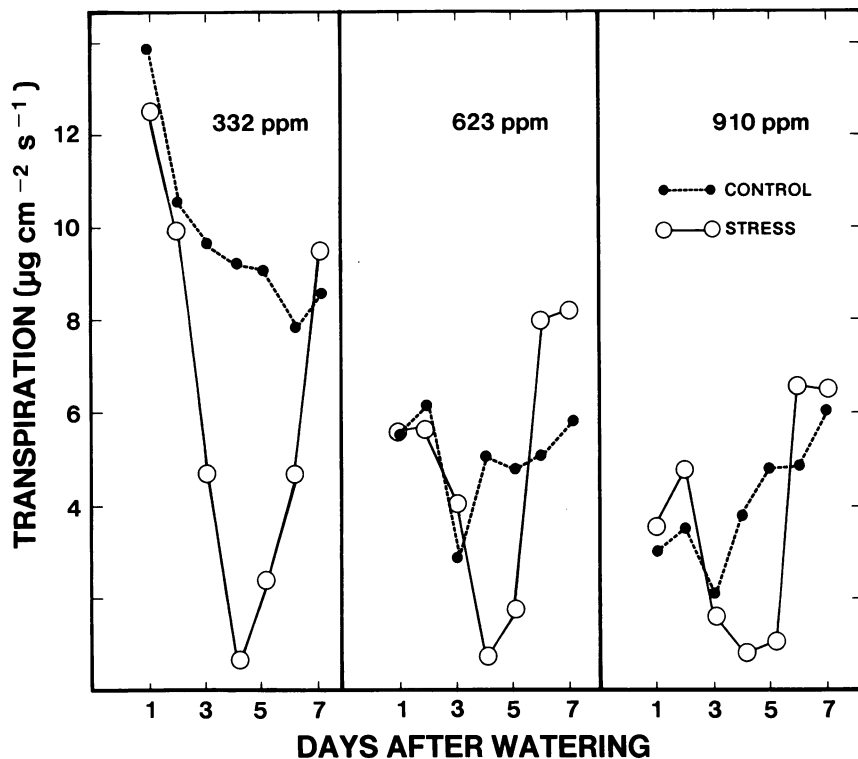


FIG. 4. Mean transpiration rate on d 4 of the stress treatment measured on the third trifoliolate leaf of soybeans grown at low ($332 \mu\text{l l}^{-1}$), intermediate ($623 \mu\text{l l}^{-1}$), and high ($910 \mu\text{l l}^{-1}$) CO₂ concentrations in field chambers. $n = 10$.

higher than the corresponding control plants (Fig. 4).

Transpiration rates reached essentially identical low values on day 4 of the stress for all CO₂ treatments (Fig. 4) in spite of large differences in stomatal resistance (Fig. 3) because resistances higher than 10 to 20 s cm^{-1} had little effect on transpiration (Fig. 5). Although the use of resistance arises naturally by analogy with electric circuitry (13), the units of conductance may be preferred since the relationship of conductance to transpiration is linear. Also, small differences in resistance, observed early in the stress week when resistance values were low and water potentials were high, become large differences in conductance and, therefore, the rate of water use, as in Figure 1.

The data in Figure 6 show that in leaves of the well watered plants, high atmospheric CO₂ increased the concentration of starch from 280 (ambient chamber, $322 \mu\text{l l}^{-1}$) to 360 mg g^{-1} dry weight (at $623 \mu\text{l l}^{-1}$) and to 325 mg g^{-1} dry weight (at $910 \mu\text{l l}^{-1}$). The sugar concentration of these nonstressed plants was

unaffected by CO₂. Water stress treatment in the open plot and ambient chamber decreased the starch concentration 28 and 37%, respectively, and significantly ($P = 0.05$) increased the sugar concentration. At the intermediate CO₂ treatment, starch was decreased only 15%, and similar sugar concentrations occurred. At the $910 \mu\text{l l}^{-1}$ CO₂ treatment level, no significant differences were observed in either starch or sugar concentrations.

DISCUSSION

Increased efficiency of water use in dry matter production may be the most significant direct effect on crop plants of the rapidly increasing atmospheric CO₂ concentration. Decreased transpiration rates have frequently been measured on individual leaves of crop plants under conditions of elevated CO₂ (5, 6, 15, 22). In the present experiment, whole plants grown in high CO₂

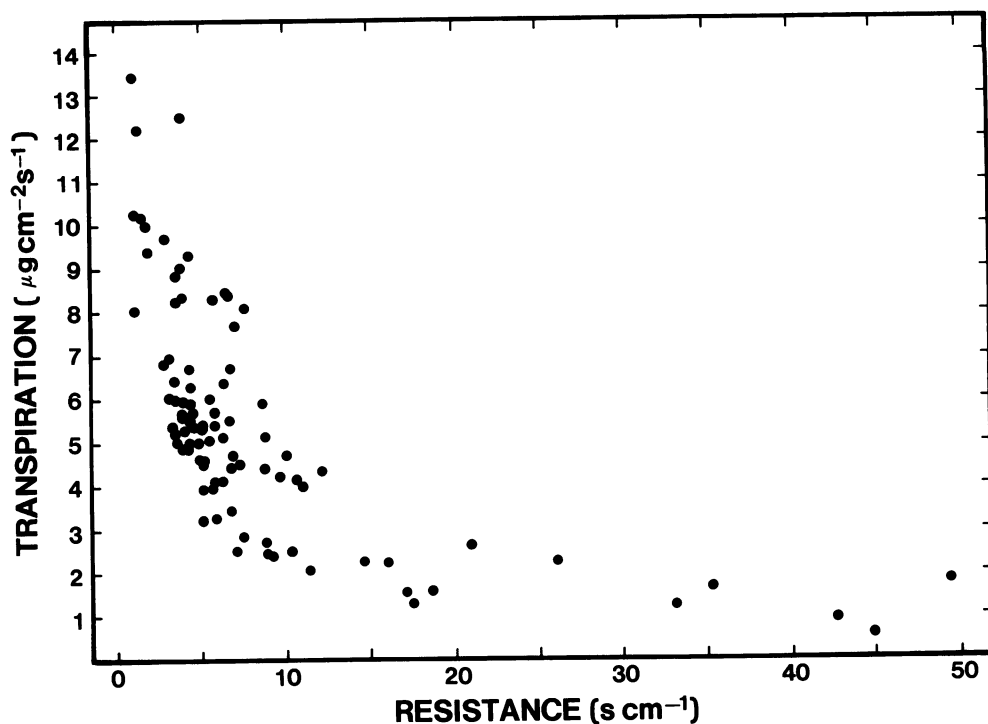


FIG. 5. Transpiration rate as a function of stomatal resistance of soybean plants grown in field chambers at six CO_2 concentrations. Each point represents the mean of 10 measurements of transpiration and conductance made throughout the stress and recovery period as described in "Materials and Methods."

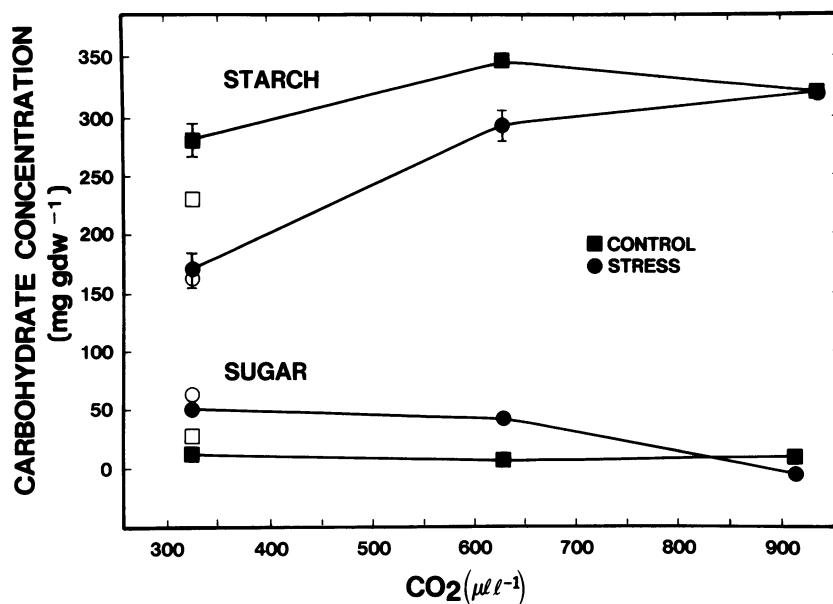


FIG. 6. Mean starch and sugar content of leaf discs collected the afternoon of day 4 of the stress treatment from the third, fourth, and fifth trifoliolate leaves of soybeans grown at low ($332 \mu\text{l l}^{-1}$), intermediate ($623 \mu\text{l l}^{-1}$), and high ($910 \mu\text{l l}^{-1}$) CO_2 concentrations in field chambers. Bars, SE from ANOVA where SE is larger than symbol; $n = 6$.

atmospheres, although larger, transpired less rapidly and conserved more soil moisture than ambient level or low CO_2 grown plants in pots of equal volume. Consequently, during a water stress, leaves of low CO_2 plants would show higher leaf resistance and lower photosynthetic carbon fixation than high CO_2 plants causing a greater drain on leaf carbohydrate reserves. These starch and sugar values are similar to those published by Mauney *et al.* (10) for 'Bragg' soybeans grown at 330 and $630 \mu\text{l l}^{-1}$ CO_2 in a glass house under full sunlight. A reduction in starch accumulation and an increase in sugar concentration at low leaf water potential similar to those reported here for the ambient level CO_2 treatment were previously reported for 'Bragg' soybeans grown in the field (21). The increased sugars and moderately reduced starch observed in the $623 \mu\text{l l}^{-1}$ CO_2 treatment indicated an intermediate degree or duration of the stress. Although the decrease in transpiration of the $910 \mu\text{l l}^{-1}$ CO_2 plants at 4 and 5 d after watering indicated the onset of water stress,

there had been as yet no drain on leaf carbohydrate reserves. Thus, water stress was avoided in proportion to the CO_2 added to ambient level.

Boyer (3), Neumann *et al.* (12), and Sionit and Kramer (17), in soybean studies in controlled environment chambers at ambient or near ambient CO_2 concentration, observed stomatal closure at -0.8 to 1.2 MPa. Turner *et al.* (21) suggested that the lower critical water potential for stomatal closure of -1.5 to -1.7 MPa measured in their field experiments could be due to greater light, the larger diurnal gradients of water potential and the more gradual development of plant water deficits found in field conditions. Under our conditions, the water potential at which stomatal closure occurred (or at which conductance values reached about 0.05 cm s^{-1}) ranged from about -0.8 MPa for high CO_2 plants to -1.4 MPa for low CO_2 plants (Fig. 7). Well watered plants showed little difference in water potential due to CO_2 (Fig. 2) and at a given water potential, conductance was

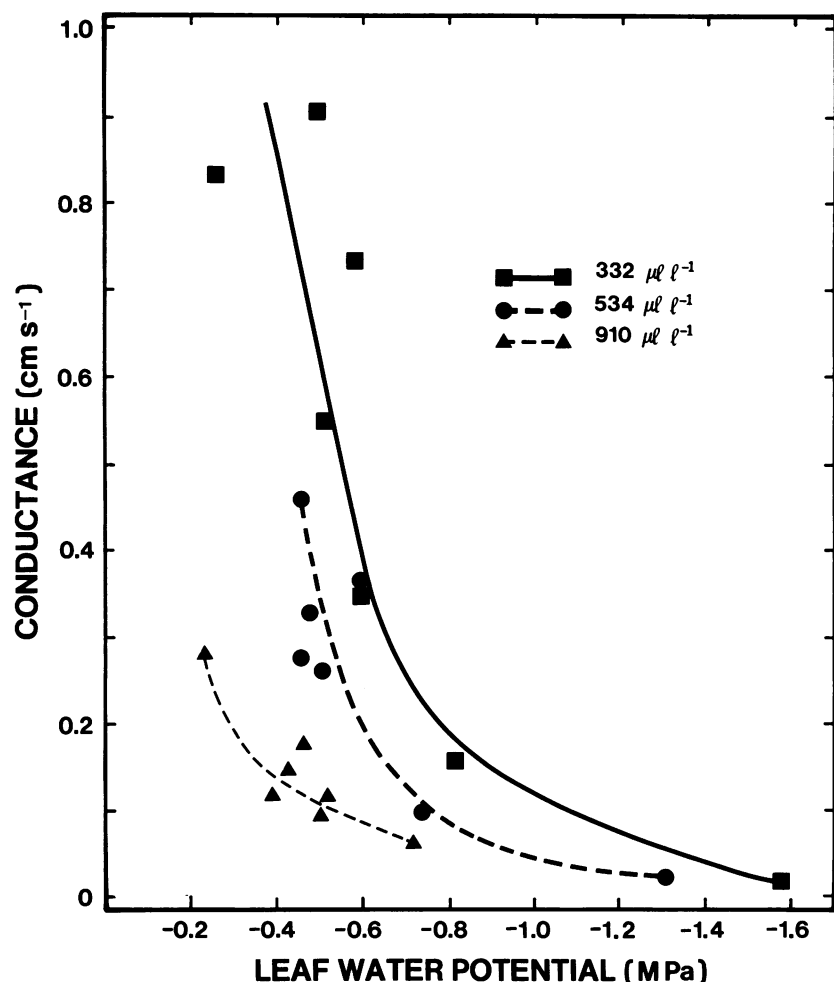


FIG. 7. Stomatal conductance as a function of water potential of soybean plants grown in field chambers at low (332 $\mu\text{l l}^{-1}$), intermediate (534 $\mu\text{l l}^{-1}$), and high (910 $\mu\text{l l}^{-1}$) CO₂ concentrations. Each point represents the mean of four water potential measurements and 10 conductance measurements as described in "Materials and Methods." Connecting lines were drawn by eye.

higher for low CO₂ plants (Fig. 7). These findings contrast with the observations of Sionit *et al.* (18) that under well watered (controlled environment) conditions, wheat plants grown at 1000 $\mu\text{l l}^{-1}$ typically showed water potentials approximately 0.2 MPa lower than plants grown at 350 $\mu\text{l l}^{-1}$ CO₂, and at a given water potential conductance values were higher for high CO₂ plants, *i.e.* the stomates of high CO₂ plants closed at a lower water potential than those of low CO₂ plants. This contrast is probably due to the ability of wheat plants to generate osmoticum as leaf water potentials drop, especially for high CO₂ plants, thereby maintaining sufficient turgor to keep stomates more open (18). Thus, although soybeans grown at elevated CO₂ concentrations tend to avoid water deficits through water conservation as demonstrated here, from the data in Figure 7, it can be inferred that increased CO₂ does not improve their ability to maintain turgor (and therefore carbon fixation capacity) as leaf water deficits develop.

In this experiment, the increase in leaf area at high CO₂ concentration shown in Figure 1 was not sufficient to counteract the lower rate of transpiration per unit leaf area; hence, water use per plant declined. However, this result may not pertain to other species of plants (B. Strain, personal communication) or to soybeans grown in conventional rows. Experiments in open top field chambers are therefore planned to investigate the influence of CO₂ enrichment on water use and soil-plant water relations of soybeans growing in conventional rows.

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